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Daily production and photosynthetic characteristics of *Nostoc flagelliforme* grown under ambient and elevated CO_2 conditions

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Abstract

Diurnal photosynthesis of *Nostoc flagelliforme* was investigated at varied levels of CO_2 concentrations and desiccation in order to estimate the effects of enriched CO_2 and watering on its daily production. Photosynthetic activity was closely correlated with the desiccated status of the algal mats, increased immediately after watering, reached a maximum at moderate water loss, and then declined with further desiccation. Increased CO_2 concentration enhanced the diurnal photosynthesis and raised the daily production. Watering twice per day enhanced the daily production due to prolonged period of active photosynthesis. The values of daily net production were 132–1280 μmol CO_2 g (d. wt)$^{-1}$ d^{-1}, corresponding to about 0.6–6.1% daily increase in dry weight. High-CO_2-grown mats required higher levels of photon flux density to saturate the alga's photosynthesis in air. Air-grown mats showed higher photosynthetic affinity for CO_2 and higher levels of dark respiration compared with high-CO_2-grown samples.

Abbreviations: [CO_2] – CO_2 concentration, $D_{1/2}$ – half-period for desiccation, $K_{0.5}$ (CO_2) – apparent photosynthetic affinity for CO_2, RWC – relative water content

Introduction

Nostoc species often colonize environments that are extreme in conditions such as the amount of heat or cold, moisture, salinity, alkalinity, acidity, or radiation. The terrestrial desiccation-tolerant form, *Nostoc flagelliforme*, is distributed widely, though mainly in semi-desert regions (Gao 1998). In China, it is distributed in the northern and west-northern parts, covering eight provinces, Xinjiang, Inner Mongolia, Qinghai, Gansu, Ningxia, Shaanxi, Shanxi and Hebei (Diao 1996). Water is an important factor limiting its distribution (Qian et al. 1989): the yearly precipitation in its habitats is usually 200–300 mm and the annual relative humidity is about 50%. Growth occurs mainly from June to September (Qian et al. 1989). During this period, the rainfall is responsible for about 60–70% of the yearly precipitation, and the dew is usually visible for about 80 days, the monthly mean temperature is about 15–20 °C, and the daily temperature difference is as big as 16 °C (Qian et al. 1989).

N. flagelliforme has the capacity to survive in a dry state for hundreds, perhaps thousands of years. It not only remains alive when air-dried, but when wetted, it absorbs water, swells, and is revitalized (Potts 2000). The recovery time for maximal photosynthetic and respiratory activities after rehydration showed dependency on the length of storage in dryness, longer storage required more time for the recovery (Scherer et al. 1984). During rehydration, its photosynthetic recovery was light-dependent (Gao et al. 1998a), correlated to the recovery of energy charge (Scherer et al. 1986), and required exogenous addition of potassium (Qiu and Gao 1999).

N. flagelliforme has been used for food delicacy or ingredients of Chinese medicine for hundreds of years, and in recent years has been the subject of research in response to the heavily reduced resources and towards possible cultivation (Gao 1998). However, little progress has been made up to now. Although the reactivated photosynthetic activity of *N. flagelliforme* is comparable to other blue-green algae (Qiu and Gao 1999), its growth is very slow, the increasing in length in culture by 19–30% in 14 days (Zhu et al. 1982), 19–43% in 12 days (Cui 1983) or 255% by weight in 58 days (Hu et al. 1987). Long-term field observations showed 6% elongation in a year (Dai 1992). However, such data are hardly informative in terms of its growth potential or daily productivity, since humidity and soil moisture were not controlled critically. The length of the algal filaments varies much according to the changes in the humidity or moisture in soil. Diurnal photosynthesis needs to be investigated to estimate its daily production and growth. The aim of the present study was to assess the influence of two factors, CO_2 enrichment and watering on its daily production.

Materials and methods

Nostoc flagelliforme (Berk. & Curtis) Bornet & Flah. was collected at Siziwangqi, Inner Mongolia, and stored dry for 2–3 years until used for experiments. Samples were rinsed 3–4 times in distilled water for 1–2 min each time and then rehydrated in BG_{11} medium (Stanier et al. 1971) at 40 μmol photon m^{-2} s^{-1} and 25 °C for 10 h for fully photosynthetic recovery (Qiu and Gao 1999). Then, the rewetted samples were spread on plastic nets as algal mats of about 9.4 cm in diameter, with the biomass density being about 5.8 mg (d. wt) cm^{-2}. The algal mats were cultured in a CO_2 chamber (E7 Conviron) for 5–6 days before their photosynthesis was measured. Samples were immersed in BG_{11} medium for half an hour each time to replenish water and nutrients. This was conducted at 0900 for those mats watered only once per day and at 0900 and 1930 for those watered twice per day. Daily changes of temperature in the CO_2 chamber are shown in Figure 1. Light was provided with cool-white fluorescent lamps. The highest irradiances were 414 and 114 μmol photon m^{-2} s^{-1} in the high and low light zones, respectively. The light/dark cycle was 14:10. CO_2 concentration was maintained at 350 or 1500 μL L^{-1} CO_2.

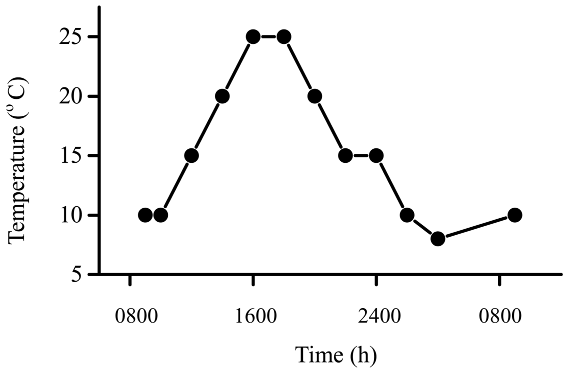

Figure 1. Daily pattern of controlled temperature during culture in the CO_2 chamber.

Photosynthetic CO_2 uptake was measured via a closed system as previously reported (Qiu and Gao 1999). The diurnal photosynthesis was instantly investigated at the same irradiance, temperature and $[CO_2]$ (CO_2 concentration) as in culture. The photosynthetic-light response (P-I) curves were determined at 25 °C and the same $[CO_2]$ as in the CO_2 chamber. The responses of photosynthetic CO_2 uptake to $[CO_2]$ were assessed at 25 °C and 770 μmol photon m^{-2} s^{-1}. Net photosynthesis (P_n) and dark respiration (R_d) (μmol CO_2 g (d.wt)$^{-1}$ h^{-1}) were determined as follows:

$$P_n \quad \text{or} \quad R_d = C/t \cdot V \cdot 273/(237 + T) \cdot 1/22.4 \cdot 1/W_d \cdot 60,$$

where C/t is the negative slope of CO_2 concentration in the closed system during data reading; V, the volume (L) of the closed system; T, temperature in the assimilation chamber (°C); W_d, the dry weight of samples (80 °C, 20–24h). The relative water content (RWC) of the mat was calculated as follows:

$$RWC = (W_t - W_d)/(W_w - W_d) \cdot 100\%,$$

where W_t is the instantaneous weight of samples; W_w, the initial wet weight.

Parameters for P-I curves were analyzed according to Henley (1993).

$$P = P_m \cdot \tanh(\alpha I/P_m) + R_d, \quad I_k = P_m/\alpha,$$

where I represents irradiance; P, photosynthetic activity at certain irradiance; P_m, light-saturated photosynthesis; I_k, saturating irradiance for photosynthesis.

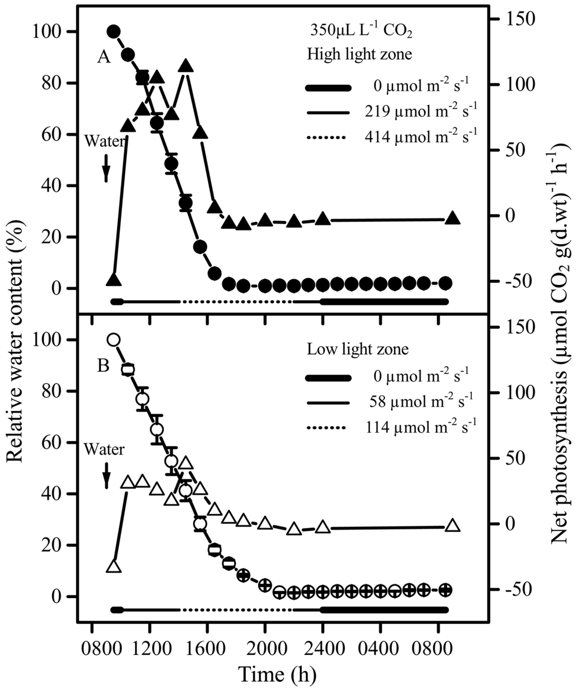

Figure 2. Diurnal photosynthetic performance and relative water content of *Nostoc flagelliforme* mats cultured at 350 μL L^{-1} CO_2, rehydrated once a day at 0900. Changes in photon flux density were controlled to follow the patterns in the high and low light zones. Photosynthetic activity was monitored under the same conditions as in culture. Circle indicates the relative water content of the algal mats (mean ± SD, n = 3); triangle indicates the net photosynthesis of the algal mats.

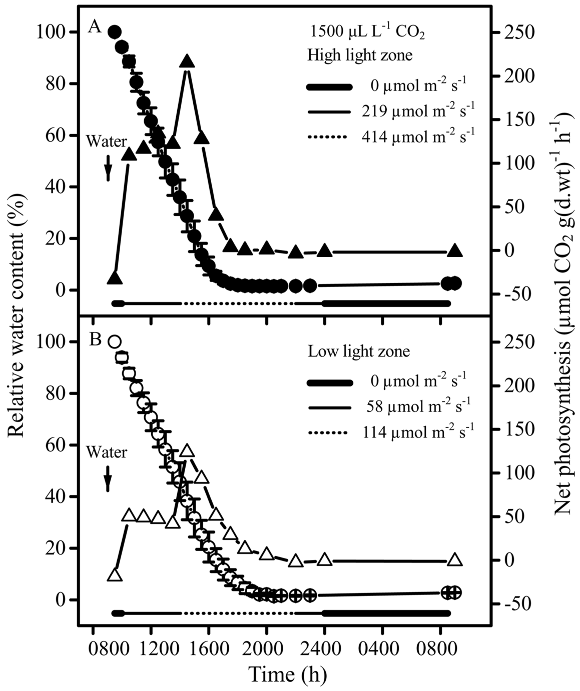

Figure 3. Diurnal photosynthetic performance and relative water content of *Nostoc flagelliforme* mats cultured at 1500 μL L^{-1} CO_2, rehydrated once a day at 0900. Other details and symbols as in Figure 2.

The ascending slope at limiting irradiances, α, was calculated to assess the photosynthetic efficiency. Responses of photosynthetic CO_2 uptake to [CO_2] were analyzed according to Michaelis-Menten equation and the parameters were estimated directly from the curve of 1/*v* versus 1/[S]. $K_{0.5}(CO_2)$, the CO_2 concentration required for half saturated photosynthesis, is used to define the apparent photosynthetic affinity for CO_2; V_{max} represents CO_2 saturated photosynthesis. Relationships between photosynthesis and environmental factors (light, [CO_2], RWC, temperature and watering) were analyzed according to correlation analysis (simple correlation and partial correlation) and regression analysis (multiple regression and stepwise regression).

Results

The RWC of algal mats after watering decreased with time (Figures 2, 3, 4 and 5). Half-periods for desiccation ($D_{1/2}$) after the first watering were 3.7 ± 0.3 and 4.1 ± 0.1 h in high and low light zones, respectively. It seemed that algal mats were desiccated slightly faster in high light zone. Desiccation after the second watering was always faster than the first. The $D_{1/2}$ values after the first and second watering were respectively 3.7 and 1.9 h in high light zone and were respectively 4.0 and 2.5 h in low light zone. Compared with the first desiccation, temperature and light levels were higher in the initial phase of the second. Algal desiccation was less affected by irradiance than by temperature. The difference in $D_{1/2}$ between low and high light zones was less than 0.6 hour. Thus, the increased rate of desiccation after the second watering was mainly associated with an increase in temperature.

The photosynthetic activity of *N. flagelliforme* in culture was closely correlated with light, desiccation and [CO_2] (Figures 2, 3, 4 and 5). The simple correlations for these factors with net diurnal photosynthesis were significant ($P < 0.05$) and the partial correlations for them were larger than those with other factors (temperature and watering times per day) (Table 1). All the coefficients for multiple and stepwise regression between these factors (light, [CO_2] and

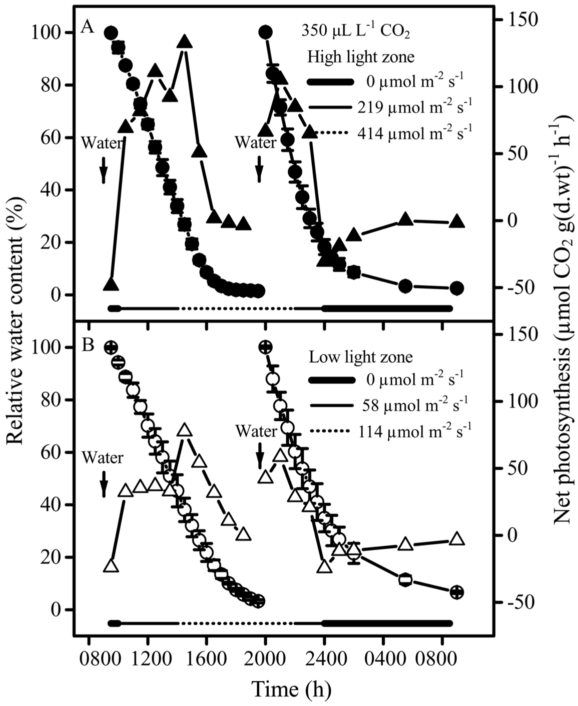

Figure 4. Diurnal photosynthetic performance and relative water content of *Nostoc flagelliforme* mats cultured at 350 μL L^{-1} CO_2, rehydrated twice per day at 0900 and 1930. Other details and symbols as in Figure 2.

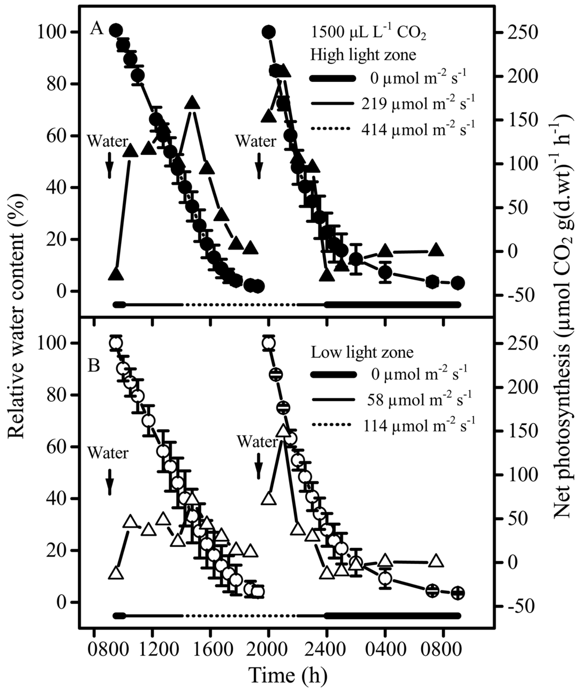

Figure 5. Diurnal photosynthetic performance and relative water content of *Nostoc flagelliforme* mats cultured at 1500 μL L^{-1} CO_2, rehydrated twice per day at 0900 and 1930. Other details and symbols as in Figure 2.

RWC) with net diurnal photosynthesis were significant ($P < 0.05$). After watering, regardless of the first or the second one, the photosynthetic activity recovered rapidly, increased to a maximum rate at a moderate water loss, then decreased with further desiccation. A peak value appeared at 1400 when irradiance was the highest. The photosynthesis of algal mats cultured in high or low light zones showed similar responses to water loss from them with the elapse of time (A *vs.* B in Figures 2, 3, 4 and 5). However, the algal mats maintained higher net photosynthetic rates in the high light zone compared to the low light zone, where the photosynthesis was light-limited. This was also true when CO_2 concentration and watering times were increased. The dark respiratory activities were higher for those cultured in the high light zone than in the low light zone. The daily net production in the low light zone was about 46% of that in the high light zone (Table 2).

The net photosynthesis was enhanced significantly ($P < 0.05$) at 1500 μL L^{-1} CO_2 compared with that at 350 μL L^{-1} CO_2 (Figure 2 *vs.* Figure 3 and Figure 4 *vs.* Figure 5). However, dark respiration was suppressed at the elevated [CO_2]. In the high light zone, the daily net production of algal mats was enhanced at 1500 μL L^{-1} CO_2 by about 98% with once daily watering and about 68% with twice daily watering; in the low light zone, the enhancement was about 266% and 62% with once and twice daily watering, respectively (Table 2). Light-saturated photosynthesis for high-CO_2-grown samples (322 μmol CO_2 g(d.wt)$^{-1}$ h^{-1}, measured at 1500 μL L^{-1} CO_2) was much higher than that for air-grown samples (199 μmol CO_2 g (d.wt)$^{-1}$ h^{-1}, measured at 350 μL L^{-1} CO_2); and the saturating irradiance (I_k) for photosynthesis was markedly higher in high-CO_2-grown samples (Figure 6). The values of I_k were 167 and 402 μmol photon m^{-2} s^{-1} at 350 and 1500 μL L^{-1} CO_2, respectively, indicating that higher irradiance was required to saturate the photosynthesis of *N. flagelliforme* at elevated [CO_2]. Air-grown samples showed higher levels of dark respiration compared to high-CO_2-grown samples. The photosynthesis was not saturated at present atmospheric CO_2 level for both high-CO_2-grown and air-grown mats (Figure 7). Air-grown samples showed a higher photosynthetic CO_2 affinity ($K_{0.5}(CO_2)$ = 346 μL L^{-1}) than high-CO_2-grown ones ($K_{0.5}(CO_2)$ = 421 μL L^{-1}), indicating the alga's acclimation to the CO_2 levels in culture. CO_2-

Table 1. Coefficients of simple correlation (data set below diagnal line) and partial correlation (data above diagonal line) among the net photosynthetic rates of *Nostoc flagelliforme* (P), light (L), [CO2], relative water content (RWC), temperature (T) and watering times per day (W.T.). The correlation coefficients were derived from data in Figures 2, 3, 4 and 5. Values in parentheses less than 0.05 indicate significant correlations.

	P	L	[CO_2]	RWC	T	W.T.
P		0.462	0.271	0.439	0.064	0.062
L	0.497 (0.000)		-0.121	-0.088	0.525	-0.031
[CO_2]	0.205 (0.019)	0.000 (1.000)		-0.135	-0.026	-0.016
RWC	0.318 (0.000)	-0.115 (0.188)	-0.016 (0.853)		-0.348	0.040
T	0.234 (0.007)	0.624 (0.000)	-0.002 (0.980)	-0.354 (0.000)		-0.072
W.T.	0.048 (0.584)	-0.071 (0.416)	0.000 (1.000)	0.114 (0.192)	-0.124 (0.155)	

Table 2. Daily production of *Nostoc flagelliforme* based on data in Figures 2, 3, 4 and 5 and estimated as accumulated diurnal net photosynthesis.

CO_2 concentration (mL L^{-1})	Watering times (d^{-1})	Daily production (μmol CO_2 g (d. wt)$^{-1}$ d^{-1}) High light zone	Low light zone
350	once	416	132
350	twice	762	356
1500	once	825	484
1500	twice	1280	576

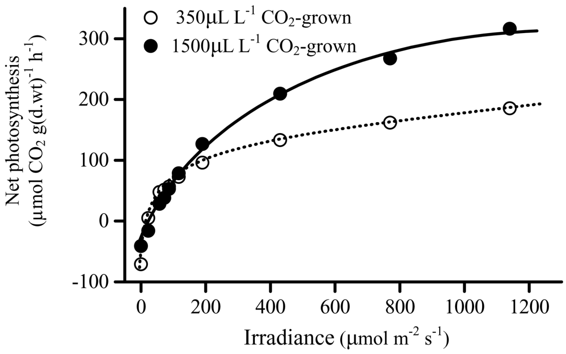

Figure 6. Photosynthetic activity as a function of irradiance for fully rehydrated *Nostoc flagelliforme*. Samples had been cultured for 5-6 days in high light zone at 350 and 1500 μL L^{-1} CO_2. Photosynthetic measurements were carried out at 25 °C and the same [CO_2] as in culture.

saturated photosynthesis (V_{max}) was similar in the algal mats cultured at the varied levels of CO_2.

There was little difference in the maximal net photosynthesis of algal mats with once or twice daily watering (Figures 2, 3, 4 and 5). However, watering influenced the daily production of algal mats through prolonging the period for active photosynthesis. The period for positive photosynthesis was 7–11 h per day with once daily watering, while it was 11–13 h per day with twice daily watering. The daily net production increased by an average of 81% with twice daily watering compared with once daily watering (Table 1). The RWC of algal mats with twice daily watering was about 20–30% at the beginning of dark cycle, and longer light period would further benefit their daily net production.

Discussion

Water is indispensible for the maintenance of cell integrity and function (Potts 1999). The balance of gain and loss of water is important for *N. flagelliforme* to survive in nature. Desiccation of this alga was suggested to be important against disintegration by bacteria (Wang et al. 1992). Increased watering enhanced the daily production via prolonging the period for

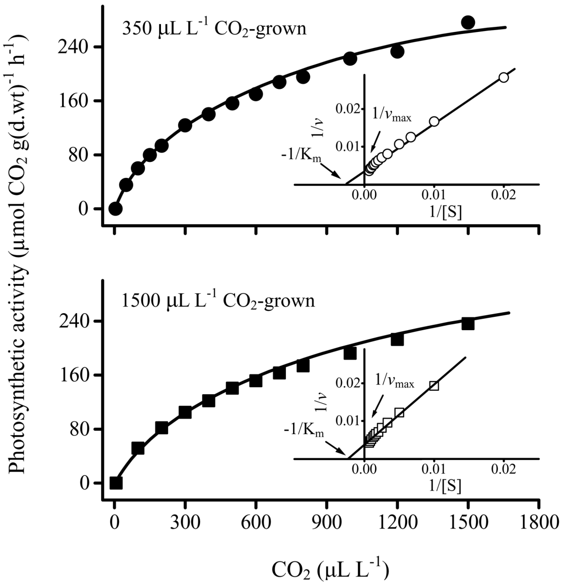

Figure 7. Photosynthetic CO_2 assimilation as a function of CO_2 concentration for fully rehydrated *Nostoc flagelliforme*. Samples had been cultured for 5–6 days in high light zone at 350 and 1500 μL L^{-1} CO_2. Photosynthetic activity was measured at 25 °C and 770 μmol photon m^{-2} s^{-1}.

positive photosynthesis. However, the upper limit needs to be set to avoid its disintegration.

N. flagelliforme could maintain positive photosynthesis for 7–11 hours per day with once daily watering. In general, the length of this period depends on the water status of the mat, this being influenced by wind, humidity, temperature and solar radiation. The culture conditions used for this study are close to those in its habitats during the main growth period (June to September) except for the lack of wind. Wind is frequent in the habitats of *N. flagelliforme* in summer, playing an important role in accelerating water loss from the colonies (Gao et al. 1998b). Thus, the daily period for this organism to maintain positive photosynthesis in nature is probably shorter than in the laboratory, assuming that both materials receive the same amount of water.

Daily net production of *N. flagelliforme* was 132-1280 μmol CO_2 g $(d.wt)^{-1}$ d^{-1} under culturing (Table 1). The organic carbon content is about 25% (Gao 1998). Consequently, the daily net production corresponds to 6–61 mg (d.wt) g $(d.wt)^{-1}$ $d^{-1\prime}$; that is, 0.6–6.1% daily increase by weight. Cui (1985) has shown that the *in situ* daily net production of *N. flagelliforme* in July was 14 mg CO_2 g (d. $wt)^{-1}$ d^{-1}, corresponding to about 1.5% increase by weight per day. The daily net production of *N. flagelliforme* cultured under ambient CO_2 is comparable with this and the other data quoted in the Introduction, assuming that filament elongation is proportional to the increase of dry matter.

Photosynthetic CO_2 affinity of *N. flagelliforme* in culture was greatly affected by CO_2 concentration and air-grown samples possessed a higher photosynthetic CO_2 affinity than high-CO_2-grown ones (Figure 7). This is consistent with reports for some aquatic blue-green algae (Kaplan and Reinhold 1999), which can photosynthesize at very low CO_2 concentration because they possess the CO_2-concentrating mechanism and can raise markedly the CO_2 concentration around the carboxylase (Kaplan and Reinhold 1999). Low CO_2 compensation point and reductive apparent photorespiration suggest that a similar CO_2-concentrating mechanism occurs in *N. flagelliforme* (Qiu and Gao 2001). This study has made it clear that the photosynthetic activity of *N. flagelliforme* is not saturated at the present atmospheric CO_2 level (350 μL L^{-1}). Any natural or artificial increase in [CO_2] would benefit photosynthetic production and growth of the alga.

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